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Chamber formation leads to Mg/Ca banding in the planktonic foraminifer *Neoglobobulimina pachyderma*

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Keywords: Mg/Ca paleothermometry, *Neoglobobulimina pachyderma*, planktonic foraminifera, intratest Mg/Ca variability, sediment trap.

Highlights:

- Large amplitude Mg/Ca variability (<0.1-7 mmol/mol) at constant temperatures
- High Mg/Ca bands deposited on outside of laminae at the end of chamber formation
- Crust Mg/Ca similar to laminar calcite and biologically controlled

- Temperature influence on Mg/Ca despite large intra- and interest variability

Abstract

Many species of planktonic foraminifera show distinct banding in the intratest distribution of Mg/Ca. This heterogeneity appears biologically controlled and thus poses a challenge to Mg/Ca paleothermometry. The cause of this banding and its relation with chamber formation are poorly constrained and most of what we know about intratest Mg/Ca variability stems from culture studies of tropical, symbiont-bearing foraminifera. Here we present data on the non-spinose, symbiont-barren *Neogloboquadrina pachyderma* from the subpolar North Atlantic where wintertime mixing removes vertical gradients in temperature and salinity. This allows investigation of biologically controlled Mg/Ca intratest variability under natural conditions. We find that intratest Mg/Ca varies between <0.1 and 7 mmol/mol, even in winter specimens. High Mg/Ca bands occur at the outer edge of the laminae, indicating reduced Mg removal at the end of chamber formation. Our data thus provide new constraints on the timing of the formation of such bands and indicates that their presence is intrinsic to the chamber formation process.

Additionally, all specimens are covered with an outer crust consisting of large euhedral crystals. The composition of the crust is similar to the low Mg/Ca bands in the laminar calcite in winter and summer specimens, indicating a tight biological control on crust formation and composition. Nevertheless, despite high intratest variability, the median Mg/Ca of summertime tests is higher than that of wintertime tests. This provides support for Mg/Ca paleothermometry, but to improve the accuracy of paleotemperature estimates biological effects on Mg incorporation need to be better accounted for.

1. Introduction

The Mg/Ca ratio in foraminiferal calcite is widely used to reconstruct seawater temperatures (Barker et al., 2005; Jonkers et al., 2010b; Lea et al., 2000; Mashiotta et al., 1999). However, Mg/Ca-temperature relationships are species specific and remain (largely) empirical and many studies have shown a tight biological control on Mg incorporation in foraminiferal calcite (Anand et al., 2003; Bentov and Erez, 2006; de Nooijer et al., 2014; Jonkers et al., 2012; Jonkers et al., 2013a; Lea et al., 1999; Spero et al., 2015). In particular, the Mg distribution within single foraminifera tests shows large, non-temperature related variability (Eggins et al., 2003; Eggins et al., 2004; Kunioka et al., 2006; Sadekov et al., 2005). Accurate paleotemperature reconstructions thus require a solid understanding of the nature of and controls on such variability.

Magnesium concentrations in foraminiferal calcite are orders of magnitude lower than in seawater and thus indicate active discrimination against the incorporation of Mg during calcification (e.g. Bentov and Erez, 2006; de Nooijer et al., 2014). Planktonic foraminifera build their skeleton by progressively adding chambers. In the ideal case, each time a new chamber is added the older chambers are covered by an additional layer of calcite (Erez, 2003; Hemleben et al., 1977; Reiss, 1957), resulting in a laminated structure. Mg/Ca (and other trace element) banding has been observed in this laminar calcite (Eggins et al., 2004; Erez, 2003; Hathorne et al., 2009; Kunioka et al., 2006; Sadekov et al., 2005). Yet, this intratest Mg/Ca heterogeneity is too large to be caused by temperature variability experienced during the growth of the foraminiferal test and Eggins et al. (2004) attributed such banding in the final chamber of *Orbulina universa* to pH changes as a result of diurnal changes in endosymbiont activity. However, similar banding was later also observed in symbiont-barren species, indicating that other mechanisms are required (Hathorne et al., 2009). Some researchers have suggested that Mg-banding results from the presence of organic layers in the calcite lattice (Kunioka et al., 2006), but in this study both the number of high Mg/Ca bands as well as their position do not appear to match with the organic layers. Others have also shown that high Mg/Ca bands do not occur at the location of organic layers, rendering this explanation for intratest trace element banding unlikely (Eggins et al., 2004; Sadekov et al., 2005; Spero et al., 2015). Alternative hypotheses involve the participation of a tiny amount of seawater (which has high Mg

concentrations) at the initiation of chamber formation, which would result in a high Mg band at the beginning (inner edge) of each lamina (de Nooijer et al., 2014), or an active role for mitochondria in Mg removal from the site of calcification, such that their reduced activity or density, possibly diurnally modulated, may cause high Mg bands (Spero et al., 2015). Nevertheless, the origin of Mg-banding is not yet fully understood and the suggested mechanisms are to a large extent based on observations from a single tropical and symbiont-bearing species *O. universa*, which forms a unique spherical outer chamber and hence differs from other species of planktonic foraminifera (Spero, 1988). To distinguish between these latter two hypotheses it is necessary to determine whether the high Mg/Ca bands are formed at the start of chamber formation or not. As such it is important to determine the intratest Mg/Ca distribution of planktonic foraminifera that undergo a typical lamellar calcification process. Various genera of planktonic foraminifera also form an outer crust at the end of their life cycle (Bé et al., 1979; Hemleben et al., 1977). In some species crust formation is related to gametogenesis (Bé, 1980). However in many Globorotaliids and Neogloboquadrinids the relationship with gametogenesis is less clear and it is thought that crusts are formed deep in the water column possibly in response to a temperature trigger (Hemleben and Spindler, 1983; Kohfeld et al., 1996; Srinivasan and Kennett, 1974). These outer crusts consist of large euhedral calcite crystals covering the laminar calcite and their function remains poorly understood. Importantly however, encrustation also presents another source of intratest Mg/Ca variability since crusts often have lower Mg/Ca ratios than the interior lamellar calcite (Bolton and Marr, 2013; Eggins et al., 2003; Fehrenbacher and Martin, 2010; Jonkers et al., 2012; Sadekov et al., 2005). This appears to be consistent with crust formation occurring at greater depths in the water column and it has consequently been suggested that single crust-bearing tests could (at least theoretically) be used to reconstruct vertical water column temperature profiles (Eggins et al., 2003; Sadekov et al., 2009). However, encrustation is variable both in thickness and composition (Jonkers et al., 2012; Steinhardt et al., 2015) and the use of single foraminifera as past tracers of water column properties relies on an improved understanding of the factors controlling crust formation.

To assess the causes and consequences of Mg/Ca banding and encrustation we investigate Mg/Ca distribution within *Neogloboquadrina pachyderma* tests at high resolution. *N. pachyderma* is a non-symbiotic species that shows normal lamellar calcification and is often used in paleoceanographic

reconstructions. The species dominates high latitude assemblages and could hence be an excellent species to serve as a cold-end paleothermometer, but the use of its Mg/Ca is confounded by encrustation and/or non-temperature related effects (Jonkers et al., 2013a; Meland et al., 2006). We use samples from a sediment trap moored in the subpolar North Atlantic Ocean (59.3° N, 39.7° W, 2750 m water depth) and exploit the unique conditions at the site to assess intratest Mg/Ca heterogeneity in the absence of temperature/environmental variability.

2. Oceanographic setting

The *in situ* temperature and salinity data from Central Irminger Sea mooring (59.7° N, 39.7° W; 45 km north of the sediment trap) shows a pronounced seasonal cycle in near surface temperature that quickly attenuates with depth (Fig. 1). At 10 m depth seawater temperatures vary from approximately 10 °C in early autumn to around 4.5 °C between January and May, when they reach values similar to those found at greater depths. Consequently, there is no, or only a negligible, vertical thermal gradient (i.e. isothermal conditions) in winter and spring (Fig. 1). Near surface salinity variability is less than 0.4 and similar to temperature, the vertical salinity gradient disappears from January onwards (Fig. 1). Moreover, wintertime convective mixing down to at least 400 m water depth most probably eliminates vertical gradients in any other environmental parameters (de Jong et al., 2012). Foraminifera living during the winter period have thus not experienced any significant temperature, salinity or other environmental variability, making this period/site a natural laboratory to study biological effects on test geochemistry.

3. Material and methods

Tests of *N. pachyderma* were picked from the 150-250 µm fraction of three sediment trap samples with 16-day collection intervals. These represent one sample from the stratified warm season flux pulse (5 to 20 September 2006; IRM4 A-01) and two samples from the isothermal cold season at the start of the spring bloom (1 to 16 April and 17 April to 2 May 2007; IRM4 A-14 and IRM4 A-15). Two tests were analysed from each sample. Taking the settling time and life span of planktonic

foraminifera into account, the foraminifera from these samples most likely formed their tests up to approximately a month before the start of the collection interval. Water column abundances of *N. pachyderma* in the Nordic and Labrador Seas are highest in the upper 200 m (Carstens et al., 1997; Kohfeld et al., 1996; Stangeew, 2001) and most calcification is likely to take place in this zone (Jonkers et al., 2010a). Specimens from the warm season samples could thus have experienced up to ~5 °C temperature variability, whereas those from spring were formed in very stable conditions with changes in temperature ≤ 0.5 °C (Fig. 1).

For an extended description of the sediment trap mooring and sample treatment see Jonkers et al. (2010a; 2013a; 2013b). All tests were cleaned using repeated brief ultrasonication in deionized water and methanol prior to embedding in Araldite 20/20 resin. Samples were left to harden at room temperature for at least 24 hrs and polished using fine sandpaper and a 0.3 μm Al_2O_3 emulsion. Scanning electron microscope (SEM) images were taken before and after embedding.

Element concentrations were measured using quantitative X-ray mapping on a JEOL 8530F field-emission electron microprobe equipped with 5 wavelength dispersive spectrometers (WDS) at the University of Bristol. The maps were acquired at 15 kV and 40 nA with a pixel size of 0.3 μm . To ensure the stability of the sample and achieve meaningful counting statistics the samples were coated with silver (Kearns et al., 2014; Smith, 1986). Ca was measured first for 10 ms on two spectrometers (a PETH and PETL crystal), Mg was measured subsequently for 300 ms on three spectrometers (2 TAP crystals and a TAPH crystal). Diopside was used as a standard for both Mg and Ca. High Mg/Ca bands of 1-2 pixel width are clearly identifiable (Fig. 2-4), suggesting the resolution to be on the order of 0.3-0.6 μm . However, X-rays are emitted from a larger area with the analytical resolution approximating 0.9 μm (calculated using PENEPM (Salvat et al., 2006) and defined as the distance over-which 75% of x-rays are emitted). This means that for any band <0.9 μm the intensity will be a convolution of the band measured and the adjacent material. The detection limit for Mg for an average of 4 pixels is 120 ppm and analytical uncertainty at Mg/Ca ratios ~4 mmol/mol amounts to ~11 % RSD. To obtain a comparable precision at lower Mg/Ca ratios more pixels counts need to be integrated. For Mg/Ca ratios around 1 mmol/mol 80 pixels (7.2 μm^2 ; approximately $\frac{3}{4}$ of the scale bar in Fig. 2-4) are required. Integrating over an area this size reduces the limit of detection to below 20 ppm and still

allows the accurate determination of the low Mg/Ca ratios of the crust and lamellar calcite since these are relatively homogenous. The maps were quantified using Calclmage (part of the Probe for EPMA software – ProbeSoftware Inc.) in which the full Armstrong-Love/Scott matrix correction (Armstrong, 1988) is applied to each pixel. To generate the Mg/Ca maps, pixels with Ca wt.% below 35 were masked and negative Mg values were substituted with half of the minimum value above zero. For the map in Fig. 4E no reliable Ca data were available due to beam damage of the test surface and in this case a Ca content of 40 wt.% is assumed, in line with the calcite composition of the tests.

4. Results

All tests used in this study were encrusted, showing large euhedral crystals on the outside of the test surface (Fig. 2-4). Encrustation is not always homogeneous across the test and the older chambers are often covered with a denser crust consisting of larger crystals. Tests from the cold season have generally lower Mg/Ca than those from the warm season (median values: 0.9 and 2.1 mmol/mol, respectively) and all tests, including those from the cold season, have highly variable Mg/Ca ratios, ranging between <0.1 and >7 mmol/mol (Fig. 2-5). In general, Mg/Ca ratios in the chambers of the initial whorl appear to be higher than in the subsequent chambers (although this might in part be due to relief; Fig. 3E; 4F and 4G).

All tests show thin (1-2 μm) high Mg/Ca bands within the lamellar calcite (Fig. 2-4; it should be noted that the very high Mg/Ca bands at the outside of the tests must be considered with caution as these may be due to edge effects). The distribution of Ca in the tests is homogeneous and does not show any sign of the banding that is present in the Mg/Ca (supl. fig). Older chambers often show multiple high Mg/Ca bands, with variable Mg/Ca ratios (~2-7 mmol/mol; Fig. 2-4). Importantly, all high Mg/Ca bands occur at the outer edge of the laminae, which can most clearly be seen in test 15_2 where the band extends along the outer edge of the laminar calcite and is covered by a crust on the outside of the test (Fig. 2G and 2I, and also in 15_1 (Fig. 2E and 2F) and 14_2 (Fig. 3I)). We observe no consistent difference in the Mg/Ca ratio of the bands between the warm and cold season.

The outer crust is relatively homogeneous and compositionally similar, if not identical to the low Mg/Ca bands of the lamellar calcite (Fig. 2-4). This pattern is clearest in tests from the cold season,

but is also present in specimens from the summer season, where in some cases the Mg/Ca ratio of the crust is even higher than that of the lamellar calcite (Fig. 4 and 6). Two specimens (14_2 and 01_2) suggest layering within the crust, as thin bands of higher Mg/Ca are present at the very outer edge of the test (Fig. 3I and 4H).

5. Discussion

The data presented here demonstrate a more complex distribution of Mg/Ca in the tests of Neogloboquadrinids than found in previous studies, which showed a simple two-layered structure with a high Mg/Ca inner layer and a low Mg/Ca crust on the outside of the tests (Eggins et al., 2003; Jonkers et al., 2012; Sadekov et al., 2005; Steinhardt et al., 2015). These studies attributed this simple layering to encrustation taking place at greater depths due to downward migration during ontogeny. Only Fehrenbacher and Martin (2010) reported the presence of complex layering similar to our observations in *N. dutertrei*, but they did not discuss its origin.

Assuming the generally accepted Mg/Ca temperature sensitivity of $\sim 10\%/^{\circ}\text{C}$ (Anand et al., 2003; Elderfield and Ganssen, 2000) the intratest range in Mg/Ca we observe in both warm and cold season tests would translate to a temperature range of $>40^{\circ}\text{C}$, which is clearly unrealistic. The presence of large amplitude Mg/Ca banding in cold season tests that experienced negligible temperature variability also rules out that the banding directly results from vertical water column migration even if the sensitivity would be higher. Given the generally short (~ 1 month) life span of planktonic foraminifera (Hemleben et al., 1989; Jonkers et al., 2015; Volkmann, 2000), temporal seawater temperature variability can also be discounted as a driver of the intratest Mg/Ca heterogeneity. In addition, the cold season specimens are highly unlikely to be derived from surviving remnants of the previous summer population as we observe that the test flux starts to increase during the collecting interval (Fig. 1) and stable oxygen and carbon isotopes measured in co-collected specimens suggest typical cold-water conditions (Jonkers et al., 2010a; Jonkers et al., 2013b). These tests thus stem from the new (spring bloom) population and given the constant environmental conditions in the water column experienced during their growth, we conclude that the intratest Mg/Ca variability reflects a

dominant biological control on Mg incorporation and is not the result of external variability in temperature, salinity or other environmental parameters.

The banding in the tests of *N. pachyderma* is only observed in the Mg/Ca and not in the Ca maps, indicating that the high Mg bands are not associated with organic layers within the calcite lattice since these would show as layers of reduced Ca intensity (Sadekov et al., 2005). Moreover, the presence of high Mg/Ca bands in the laminae covering preceding chambers (Fig. 2-4) rules out an association with the primary organic membrane (POM) since this membrane is only present in the newly formed chamber and not in the lamina covering the older chambers. Similarly, it has been shown that in *O. universa* the POM occurs in a broad band of low Mg/Ca (Eggins et al., 2004; Spero et al., 2015) and Sadekov et al. (2005) showed that high Mg/Ca bands in *Globorotalia truncatulinoides* are not associated with embedded organic layers. We can therefore reasonably conclude that the Mg/Ca banding in *N. pachyderma* is also unlikely to be result of organic layering in the test wall. As such, consistent with most recent hypotheses on Mg/Ca banding (de Nooijer et al., 2014; Spero et al., 2015), we propose that such banding is intrinsic to the calcification mechanism of planktonic foraminifera. Below we discuss Mg/Ca heterogeneity in the lamellar and crust calcite, as well as the paleoceanographic implications of this intratest compositional variability.

5.1. Mg/Ca banding in lamellar calcite

In *N. pachyderma* each lamina consists of a low Mg/Ca band of variable width (max. 10 μm) on the inside and a thin (1-2 μm) high Mg/Ca band on the outside. This pattern is visible in most tests and older chambers, in general, show (approximately) the number of bands expected from laminar growth of the test. Some chambers show fewer bands, which may result from absence of the laminae since calcification does not always strictly follow a laminar pattern, i.e. the layer from subsequent chamber does not always cover all preceding chambers and becomes thinner towards older chambers (Hemleben et al., 1977; Nehrke et al., 2013). In addition, the laminae may be too thin to be resolved using our techniques and/or merge with the previous lamina (Fig. 2E). Alternatively, since the composition of the high Mg/Ca bands varies, the contrast between the low and high Mg/Ca bands may be too small to allow distinction (see for instance 15_2, where the band is faintly visible in Fig 2H, but not in 2G even though they depict the same chamber). One or more laminae may thus be missing

from individual chambers, explaining the occasional absence of multiple bands in older chambers. Thus, we feel confident to suggest that typically each lamina ends with the deposition of a high Mg/Ca calcite band, which strongly suggests that discrimination against Mg incorporation into the calcite diminishes towards end of chamber formation.

As such our observations add important new constraints on the origin of the banding as they constrain the timing of its formation within the chamber formation process. Our results rule out the recent suggestion of the participation of a tiny amount of unaltered seawater with high Mg concentrations at the initiation of chamber formation (de Nooijer et al., 2014) and instead suggest that foraminifera are able to effectively control the composition of the calcifying fluid from the onset of chamber formation. This may point to the existence of an internal cation pool that becomes depleted in Ca during calcification, leaving a solution behind that is enriched in Mg, which is used at the final stage of chamber formation (Bentov et al., 2009; Erez, 2003). However, this process cannot reflect simple Rayleigh fractionation, as we do not observe a gradual increase in Mg/Ca from the inside to the outside of the lamina. Hathorne et al. (2009) have also shown that such a mechanism cannot account for trace element banding in other species. Moreover, the very existence of an internal cation pool has recently been contested (de Nooijer et al., 2014; Mewes et al., 2015; Nehrke et al., 2013), rendering this explanation unlikely.

Recent studies on *O. universa* have shown that the Mg/Ca banding within the final chamber reflects a diurnal rhythm, with the precipitation of a high Mg/Ca band at night (Eggins et al., 2004; Spero et al., 2015). Since *N. pachyderma* does not possess endosymbionts, it is clear that the banding in this species cannot result from pH changes as a result of varying symbiont activity (Eggins et al., 2004). While it is possible that the thin high Mg/Ca bands represent short episodes of rapid calcification, others have shown that changes in the calcification rate are unlikely to be the cause of banding in foraminiferal tests (Hathorne et al., 2009).

Our data show for the first time that each lamina ends with a high Mg/Ca band, suggesting that the banding is an integral part of the chamber formation process. It may be that the chamber formation in *N. pachyderma* is comparable to the wall thickening of the final chamber in *O. universa* and that the banding results from changes in the effectiveness of Mg removal from the location of calcification. Recent work suggested that the efficiency of Mg removal in *O. universa* could be regulated by changes

in the activity and/or density of mitochondria (Spero et al., 2015) and a similar mechanism may play a role in *N. pachyderma*. However, little is known about chamber formation in this species and there is no *a priori* reason to assume that chamber formation and/or mitochondrial activity and hence the banding is diurnally paced. Thus, while variable Mg removal by mitochondria presents a likely mechanism for the banding in *N. pachyderma*, the reduction in Mg removal may simply be intrinsic to the chamber formation process, with the mitochondrial activity related to the organism's function and energy balance.

5.2. Mg/Ca ratios of the crust calcite

All specimens investigated here are encrusted and the presence of crusts in cold season specimens, when the water column is fully mixed, argues against temperature trigger of crust formation as suggested for *N. dutertrei* (Hemleben and Spindler, 1983). The crusts appear thicker on the older chambers in the final whorl (Fig. 2-4). This has been observed before (Srinivasan and Kennett, 1974) and in *N. dutertrei* the thinner crust on the younger chambers was found to have lower Mg/Ca ratios, which was taken as evidence for gradual crust formation during late ontogeny (Jonkers et al., 2012; Steinhardt et al., 2015). Higher Mg/Ca ratios in the crust covering the youngest chambers than in the older chambers are not clearly evident in our new data, but the warm season specimens show enhanced variability in the Mg/Ca ratio of the crust. This is consistent with the suggestion of a tight biological control on crust formation and composition (Jonkers et al., 2012; Steinhardt et al., 2015). Taken together with the layering observed in the crust of specimens 14_2 and 1_2 these data highlight the need for additional studies to constrain the processes governing crust formation. Cold season specimens show no compositional difference between the Mg/Ca ratio of the crust and the low Mg/Ca lamellar calcite in (Fig. 2 and 3). This could be expected because the water column was well mixed and isothermal. However, neither during the warm season do the crusts have consistently lower Mg/Ca ratios than the lamellar calcite and in fact the opposite occurs in tests from sample 01 (Fig. 4 and 6). There is thus no clear indication that the crust is formed at greater depth, either because the foraminifera are able to control their Mg/Ca ratio independent of temperature, or because they remain at a relatively shallow depth throughout their life cycle. The latter would be consistent with the large amplitude in the stable isotope composition of samples from the same time

series (Jonkers et al., 2010a; Jonkers et al., 2013b) and with observations of encrusted tests at relatively shallow depths (Bauch et al., 1997; Kohfeld et al., 1996). Either way, our new data caution against the use of Mg/Ca in single specimens of *N. pachyderma* as a simple water column temperature profiler.

5.3. Implications for Mg/Ca paleothermometry

Many studies have shown a clear relationship between Mg/Ca in foraminiferal calcite and calcification temperature (Anand et al., 2003; Elderfield and Ganssen, 2000; Nürnberg, 1995). And even though intertest variability within single species of foraminifera is large, when multiple individual tests are analysed, similar temperature relationships are observed on a population level (Haarmann et al., 2011; Marr et al., 2011; Sadekov et al., 2009). In addition, despite large variability within tests, Spero et al. (2015) observed that the Mg/Ca ratio of both the low and high Mg/Ca bands in *O. universa* increased with temperature. This again suggests that on a population level Mg/Ca and calcification temperatures are correlated, but that at the level of individual tests, biological controls may overprint the temperature signal.

Previously measured bulk solution-based Mg/Ca data from the same time series are only available for samples IRM4 A-01 and A-15 and are 1.35 and 1.21 mmol/mol, respectively, thus falling in the range of our new observations (Jonkers et al., 2013a). While these bulk Mg/Ca analyses revealed little temperature dependence of Mg/Ca, the new observations on a small number of tests suggest that warm season tests have higher Mg/Ca ratios than cold season tests (Fig. 5). However, other studies have shown large intertest Mg/Ca variability in planktonic foraminifera (e.g. Haarmann et al., 2011; Jonkers et al., 2012) and we cannot rule out that our new data on a limited number of tests fall within the range of natural variability. Nevertheless, the difference between tests grown in warm and cold water is solely due to higher Mg/Ca in the low Mg/Ca lamellar calcite and in the crust and we do not observe any consistent difference in the high Mg/Ca bands, such as observed in *O. universa* (Spero et al., 2015). Consequently, biological imprints on paleotemperature estimates would depend on the proportion of the high Mg/Ca bands with respect to the entire test volume as well as the contrast between the low and high Mg/Ca layers. In the tests analysed here, calcite with >4 mmol/mol Mg/Ca makes up <4% of the total surface of the cross section (except in 01_2). Assuming that this proportion

is representative of the entire test, it is evident that even a small contribution of high Mg/Ca calcite may increase the estimated calcification temperature, particularly at low temperatures where the slope of the calibration curve is lower. For example, given modal Mg/Ca values around 0.5-0.6 mmol/mol (comparable to test 15_1; Fig. 2), the presence of bands with an Mg/Ca ratio ~6 mmol/mol making up only 2% of the volume would increase the overall Mg/Ca ratio by ~15 %. With a temperature sensitivity of ~10%/°C this would lead to an increase in the inferred calcification temperature that is significantly larger than the combined analytical and calibration error of 1°C (Anand et al., 2003). It is therefore clear that biologically controlled changes in the Mg/Ca ratio of foraminifera can significantly affect paleotemperature estimates. At the same time, the apparent positive relation between modal Mg/Ca and temperature provides support for the use of Mg/Ca to reconstruct seawater temperatures. This dichotomy highlights one of most intriguing outstanding questions surrounding the use of trace element based foraminiferal proxies: why does the test geochemistry on a population level appear to respond to environmental conditions/stimuli, whereas inter- and intra-test variability appear (orders of magnitude) larger and virtually exclusively biologically controlled?

6. Conclusions

Wintertime vertical mixing of the water column in the central Irminger Sea renders vertical gradients in temperature, salinity and other environmental parameters negligible, making this site ideal to study planktonic foraminiferal chemistry in the natural environment. Electron microprobe analyses of *N. pachyderma* tests from a sediment trap time series reveal that:

- Mg/Ca ratios in tests of *N. pachyderma* vary between <0.1 and 7 mmol/mol in the absence of temperature or other variability. The lamellar calcite consists of layers of low Mg/Ca inner and high Mg/Ca outer bands. Occasional banding occurs in the crust that covers the outer chambers of each test.
- The presence of high Mg/Ca bands on the outside of each lamina indicates a decrease in discrimination against Mg incorporation into the calcite towards the end of chamber formation.

- The Mg/Ca banding appears intrinsic to the chamber formation process. This is consistent with a recent study (Spero et al., 2015) that surmises a role for mitochondria in the (variable) removal of Mg from the location of calcification. However, the (physiological) reason for variable mitochondrial activity remains to be established and need not necessarily be diurnally paced as in *O. universa*.
- All tests analysed in this study had a rough crystalline crust on the outside of the tests. The presence of such a crust in specimens that only experienced isothermal conditions precludes a thermal trigger for encrustation and indicates a tight biological control for crust formation.
- The Mg/Ca ratios of the crust are similar or higher than the low Mg/Ca bands in the laminar calcite. This suggests that its composition is biologically controlled and/or that the crust is formed relatively shallow in the water column. These observations argue against the use of single tests as paleotemperature profilers.
- In general tests from the warm season show higher Mg/Ca than those from the cold season, supporting Mg/Ca paleothermometry. However, large inter- and intratest Mg/Ca variability appears biologically controlled and the processes governing this variability need to be better constrained to improve the accuracy of past seawater temperature estimates.

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Figure captions

Fig. 1: temperature, salinity and *N. pachyderma* test flux (150-250 μm) in the central Irminger Sea between September 2006 and mid May 2007. The red dot in the map inset shows the locations of the moorings in the Irminger Sea southeast of Greenland. The red line in the temperature graph highlights the winter and spring period when the vertical thermal gradient ≤ 0.5 °C. The horizontal black bars at the bottom indicate the collection intervals of the samples used, with the stippled lines indicating the possible interval when the foraminifera in these samples could have been living. Specimens from IRM4 A-14 and A-15 thus experienced negligible temperature and salinity variability during their life cycle.

Fig. 2: SEM images of *N. pachyderma* tests from sample IRM4 A-15 (cold season) before and after cross sectioning as well as Mg/Ca (mmol/mol) maps of the areas indicated by the orange rectangles in the upper panels. Note the high Mg/Ca bands at the end each lamina and the absence of a compositional difference between the crust and the low Mg/Ca bands of the laminar calcite.

Fig. 3: SEM images and Mg/Ca (mmol/mol) maps for sample IRM4 A-14 (cold season). Note the apparent layering in the crust in I and J.

Fig. 4: SEM images and Mg/Ca (mmol/mol) maps for sample IRM4 A-01 (warm season). Note the lower Mg/Ca ratio in the lamellar calcite than in the crust in F (upper chamber; F-1) and G (upper chamber; F-0). The grey lines in F and G show the approximate position of the profiles shown in figure 6.

Fig. 5: Lower Mg/Ca in cold season *N. pachyderma* tests: histograms of Mg/Ca ratios from the warm and cold season. Median Mg/Ca values are indicated by the black dots.

Fig. 6: Mg/Ca profiles across the summer season tests. The location of the profiles is indicated by the grey lines in figure 4F and G; roman numbers define start and end points of the paths (Fig. 4). Note

that the Mg/Ca ratio of the crust – highlighted in grey – is higher or indistinguishable from the low Mg/Ca lamellar calcite. Errors are based on a profile width of 12 μm .

References

- Anand, P., Elderfield, H., Conte, M.H., 2003. Calibration of Mg/Ca thermometry in planktonic foraminifera from a sediment trap time series. *Paleoceanography* 18, 1050, doi:10.1029/2002PA000846.
- Armstrong, J., 1988. Quantitative Analysis of Silicate and Oxide Materials: Comparision of Monte Carlo, Zaf and F (Pz) Procedures, in: Newbury, D.E. (Ed.), *Microbeam Analysis*. San Francisco Press, San Francisco, pp. 239-246.
- Barker, S., Cacho, I., Benway, H., Tachikawa, K., 2005. Planktonic foraminiferal Mg/Ca as a proxy for past oceanic temperatures: a methodological overview and data compilation for the Last Glacial Maximum. *Quaternary Science Reviews* 24, 821-834.
- Bauch, D., Carstens, J., Wefer, G., 1997. Oxygen isotope composition of living *Neogloboquadrina pachyderma* (sin.) in the Arctic Ocean. *Earth and Planetary Science Letters* 146, 47-58.
- Bé, A.W.H., 1980. Gametogenic calcification in a spinose planktonic foraminifer, *Globigerinoides sacculifer* (Brady). *Marine Micropaleontology* 5, 283-310.
- Bé, A.W.H., Hemleben, C., Anderson, O.R., Spindler, M., 1979. Chamber formation in planktonic foraminifera. *Micropaleontology* 25, 294-307.
- Bentov, S., Brownlee, C., Erez, J., 2009. The role of seawater endocytosis in the biomineralization process in calcareous foraminifera. *Proceedings of the National Academy of Sciences* 106, 21500-21504.
- Bentov, S., Erez, J., 2006. Impact of biomineralization processes on the Mg content of foraminiferal shells: A biological perspective. *Geochem. Geophys. Geosyst.* 7, 10.1029/2005GC001015.
- Bolton, A., Marr, J.P., 2013. Trace element variability in crust-bearing and non crust-bearing *N. incompta*, P-D intergrade and *G. inflata* from the Southwest Pacific Ocean: Potential paleoceanographic implications. *Marine Micropaleontology* 100, 21-33.

Carstens, J., Hebbeln, D., Wefer, G., 1997. Distribution of planktic foraminifera at the ice margin in the Arctic (Fram Strait). *Marine Micropaleontology* 29, 257-269.

de Jong, M.F., van Aken, H.M., Våge, K., Pickart, R.S., 2012. Convective mixing in the central Irminger Sea: 2002-2010. *Deep Sea Research Part I: Oceanographic Research Papers* 63, 36-51.

de Nooijer, L.J., Spero, H.J., Erez, J., Bijma, J., Reichert, G.J., 2014. Biomineralization in perforate foraminifera. *Earth-Science Reviews* 135, 48-58.

Eggins, S., De Deckker, P., Marshall, J., 2003. Mg/Ca variation in planktonic foraminifera tests: implications for reconstructing palaeo-seawater temperature and habitat migration. *Earth and Planetary Science Letters* 212, 291-306.

Eggins, S.M., Sadekov, A., De Deckker, P., 2004. Modulation and daily banding of Mg/Ca in *Orbulina universa* tests by symbiont photosynthesis and respiration: a complication for seawater thermometry? *Earth and Planetary Science Letters* 225, 411-419.

Elderfield, H., Ganssen, G., 2000. Past temperature and $\delta^{18}\text{O}$ of surface ocean waters inferred from foraminiferal Mg/Ca ratios. *Nature* 405, 442-445.

Erez, J., 2003. The Source of Ions for Biomineralization in Foraminifera and Their Implications for Paleooceanographic Proxies. *Reviews in Mineralogy and Geochemistry* 54, 115-149.

Fehrenbacher, J., Martin, P., 2010. Mg/Ca variability of the planktonic foraminifera *G. ruber* s.s. and *N. dutertrei* from shallow and deep cores determined by electron microprobe image mapping. *IOP Conference Series: Earth and Environmental Science* 9, 012018.

Haarmann, T., Hathorne, E.C., Mohtadi, M., Groeneveld, J., Kölling, M., Bickert, T., 2011. Mg/Ca ratios of single planktonic foraminifer shells and the potential to reconstruct the thermal seasonality of the water column. *Paleoceanography* 26, PA3218, 3210.1029/2010pa002091.

Hathorne, E.C., James, R.H., Lampitt, R.S., 2009. Environmental versus biomineralization controls on the intratest variation in the trace element composition of the planktonic foraminifera *G. inflata* and *G. scitula*. *Paleoceanography* 24, 10.1029/2009PA001742.

Hemleben, C., Be, A.W.H., Anderson, O.R., Tuntivate, S., 1977. Test morphology, organic layers and chamber formation of the planktonic foraminifer *Globorotalia menardii* (d'Orbigny). *The Journal of Foraminiferal Research* 7, 1-25.

Hemleben, C., Spindler, M., 1983. Recent advances in research on living planktonic foraminifera, in: Meulenkamp, J.E. (Ed.), *Reconstruction of Marine Paleoenvironments*, pp. 141-170.

Hemleben, C., Spindler, M., Anderson, O.R., 1989. *Modern Planktonic Foraminifera*. Springer Verlag, Berlin.

Jonkers, L., Brummer, G.-J.A., Peeters, F.J.C., van Aken, H.M., De Jong, M.F., 2010a. Seasonal stratification, shell flux, and oxygen isotope dynamics of left-coiling *N. pachyderma* and *T. quinqueloba* in the western subpolar North Atlantic. *Paleoceanography* 25, PA2204; doi:2210.1002/palo.20018.

Jonkers, L., De Nooijer, L.J., Reichart, G.J., Zahn, R., Brummer, G.J.A., 2012. Encrustation and trace element composition of *Neogloboquadrina dutertrei* assessed from single chamber analyses - implications for paleotemperature estimates. *Biogeosciences* 9, 4851-4860.

Jonkers, L., Jiménez-Amat, P., Mortyn, P.G., Brummer, G.-J.A., 2013a. Seasonal Mg/Ca variability of *N. pachyderma* (s) and *G. bulloides*: Implications for seawater temperature reconstruction. *Earth and Planetary Science Letters* 376, 137-144.

Jonkers, L., Moros, M., Prins, M.A., Dokken, T.M., Andersson Dahl, C., Dijkstra, N., Perner, K., Brummer, G.J.A., 2010b. A reconstruction of sea surface warming in the northern North Atlantic during MIS 3 ice-rafting events. *Quat. Sci. Rev.*, doi:10.1016/j.quascirev.2010.1003.1014.

Jonkers, L., Reynolds, C.E., Richey, J., Hall, I.R., 2015. Lunar periodicity in the shell flux of planktonic foraminifera in the Gulf of Mexico. *Biogeosciences* 12, 3061-3070.

Jonkers, L., van Heuven, S., Zahn, R., Peeters, F.J.C., 2013b. Seasonal patterns of shell flux, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ of small and large *N. pachyderma* (s) and *G. bulloides* in the subpolar North Atlantic. *Paleoceanography* 28, 164-174.

Kearns, S., Buse, B., Wade, J., 2014. Mitigating Thermal Beam Damage with Metallic Coats in Low Voltage FEG-EPMA of Geological Materials. *Microscopy and Microanalysis* 20, 740-741.

Kohfeld, K.E., Fairbanks, R.G., Smith, S.L., Walsh, I.D., 1996. *Neogloboquadrina pachyderma* (sinistral coiling) as Paleocceanographic Tracers in Polar Oceans: Evidence from Northeast Water Polynya Plankton Tows, Sediment Traps, and Surface Sediments. *Paleoceanography* 11, 679-699.

Kunioka, D., Shirai, K., Takahata, N., Sano, Y., Toyofuku, T., Ujiie, Y., 2006. Microdistribution of Mg/Ca, Sr/Ca, and Ba/Ca ratios in *Pulleniatina obliquiloculata* test by using a NanoSIMS: Implication for the

vital effect mechanism. *Geochemistry, Geophysics, Geosystems* 7, Q12P20, doi:10.1029/2006GC001280.

Lea, D.W., Mashiotta, T.A., Spero, H.J., 1999. Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing. *Geochimica et Cosmochimica Acta* 63, 2369-2379.

Lea, D.W., Pak, D.K., Spero, H.J., 2000. Climate impact of late Quaternary equatorial Pacific sea surface temperature variations. *Science* 289, 1719-1724.

Marr, J.P., Baker, J.A., Carter, L., Allan, A.S.R., Dunbar, G.B., Bostock, H.C., 2011. Ecological and temperature controls on Mg/Ca ratios of *Globigerina bulloides* from the southwest Pacific Ocean. *Paleoceanography* 26, 10.1029/2010PA002059.

Mashiotta, T.A., Lea, D.W., Spero, H.J., 1999. Glacial-interglacial changes in Subantarctic sea surface temperature and $\delta^{18}\text{O}$ -water using foraminiferal Mg. *Earth and Planetary Science Letters* 170, 417-432.

Meland, M.Y., Jansen, E., Elderfield, H., Dokken, T.M., Olsen, A., Bellerby, R.G.J., 2006. Mg/Ca ratios in the planktonic foraminifer *Neogloboquadrina pachyderma* (sinistral) in the northern North Atlantic/Nordic Seas. *Geochem. Geophys. Geosyst.* 7, 10.1029/2005GC001078.

Mewes, A., Langer, G., Thoms, S., Nehrke, G., Reichart, G.J., de Nooijer, L.J., Bijma, J., 2015. Impact of seawater $[\text{Ca}^{2+}]$ on the calcification and calcite Mg/Ca of *Amphistegina lessonii*. *Biogeosciences* 12, 2153-2162.

Nehrke, G., Keul, N., Langer, G., de Nooijer, L.J., Bijma, J., Meibom, A., 2013. A new model for biomineralization and trace-element signatures of Foraminifera tests. *Biogeosciences* 10, 6759-6767.

Nürnberg, D., 1995. Magnesium in tests of *Neogloboquadrina pachyderma* sinistral from high northern and southern latitudes. *Journal of Foraminiferal Research* 25, 350-368.

Reiss, Z., 1957. The Bilamellidea, nov. superfam., and remarks on Cretaceous Globorotaliids. *Cushman Found. Foram. Res* 8, 127-145.

Sadekov, A., Eggins, S.M., De Deckker, P., Ninnemann, U., Kuhnt, W., Bassinot, F., 2009. Surface and subsurface seawater temperature reconstruction using Mg/Ca microanalysis of planktonic foraminifera *Globigerinoides ruber*, *Globigerinoides sacculifer*, and *Pulleniatina obliquiloculata*. *Paleoceanography* 24, 10.1029/2008PA001664.

- Sadekov, A.Y., Eggins, S.M., De Deckker, P., 2005. Characterization of Mg/Ca distributions in planktonic foraminifera species by electron microprobe mapping. *Geochem. Geophys. Geosyst.* 6, 10.1029/2005GC000973.
- Salvat, F., Llovet, X., Fernández-Varea, J.M., Sempau, J., 2006. Monte Carlo simulation in electron probe microanalysis. Comparison of different simulation algorithms. *Microchimica Acta* 155, 67-74.
- Smith, M.P., 1986. Silver Coating Inhibits Electron Microprobe Beam Damage of Carbonates. *Journal of Sedimentary Research* 56, 560-561.
- Spero, H.J., 1988. Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*. *Marine Biology* 99, 9-20.
- Spero, H.J., Eggins, S.M., Russell, A.D., Vetter, L., Kilburn, M.R., Hönisch, B., 2015. Timing and mechanism for intratest Mg/Ca variability in a living planktic foraminifer. *Earth and Planetary Science Letters* 409, 32-42.
- Srinivasan, M.S., Kennett, J.P., 1974. Secondary Calcification of the Planktonic Foraminifer *Neogloboquadrina pachyderma* as a Climatic Index. *Science* 186, 630-632.
- Stangeew, E., 2001. Distribution and Isotopic Composition of Living Planktonic Foraminifera *N. pachyderma* (sinistral) and *T. quinqueloba* in the High Latitude North Atlantic. Dissertation zur Erlangung des Doktorgrades der Mathematisch-Naturwissenschaftlichen Fakultät der Christian-Albrechts-Universität zu Kiel, 98 pp.
- Steinhardt, J., de Nooijer, L.L.J., Brummer, G.-J., Reichert, G.-J., 2015. Profiling planktonic foraminiferal crust formation. *Geochemistry, Geophysics, Geosystems* 16, 2409-2430.
- Volkman, R., 2000. Planktic foraminifers in the outer Laptev Sea and the Fram Strait - modern distribution and ecology. *The Journal of Foraminiferal Research* 30, 157-176.

Figure 1

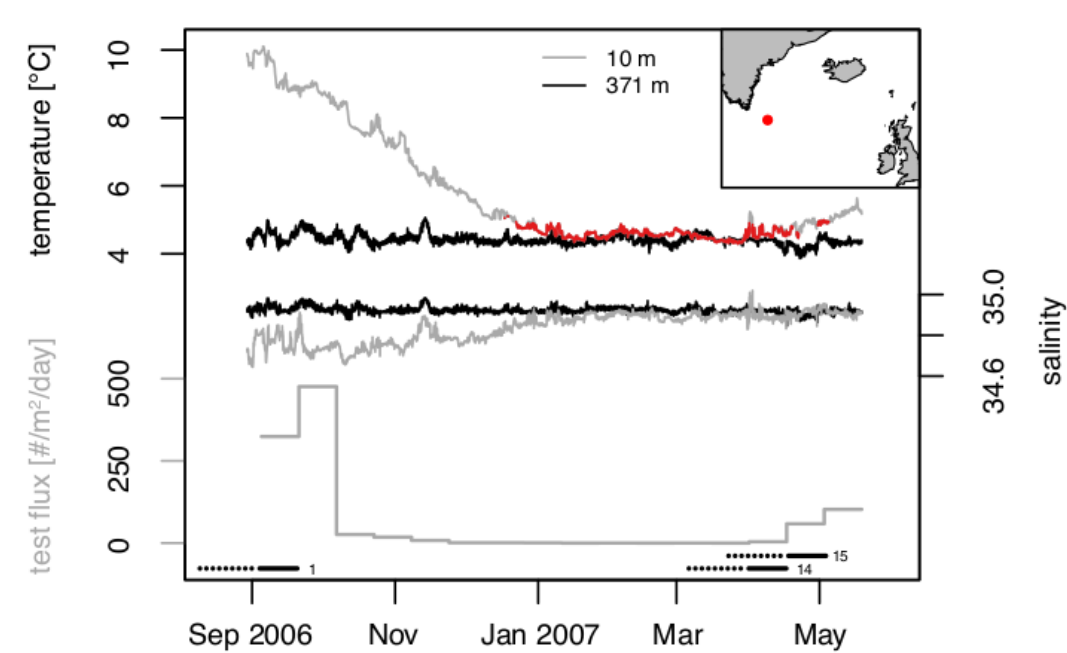


Figure 2

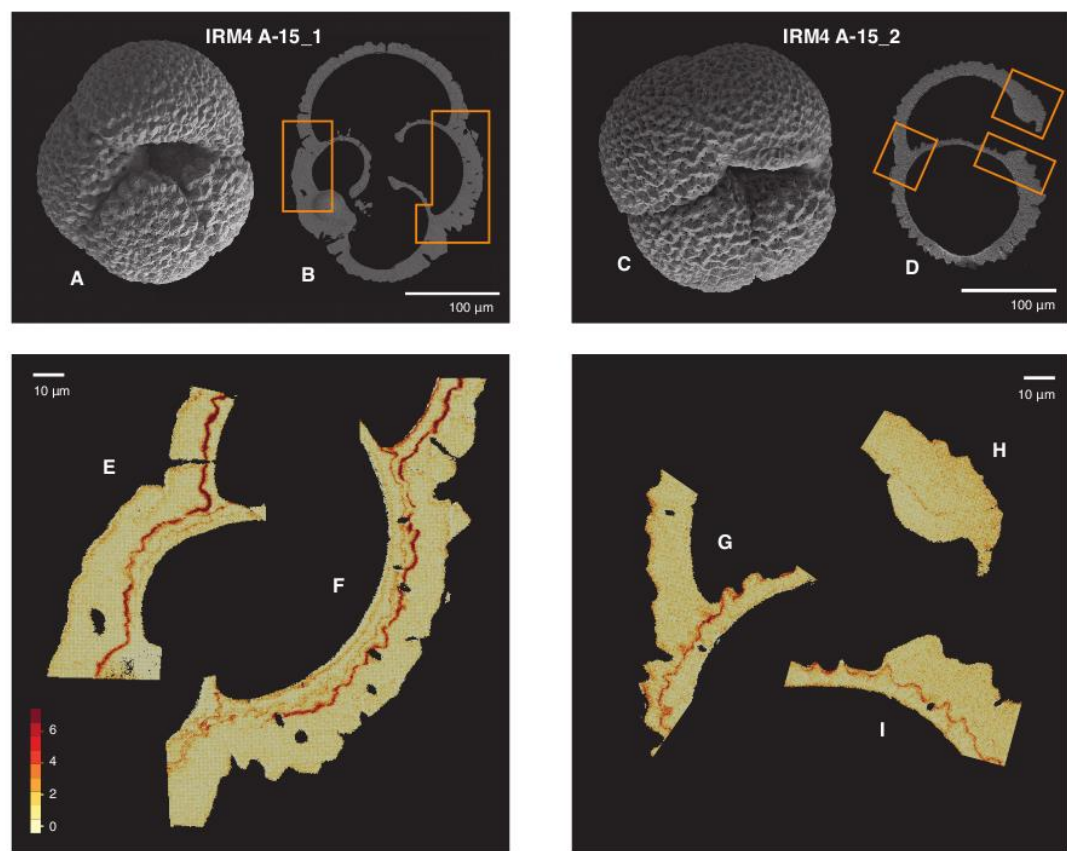


Figure 3

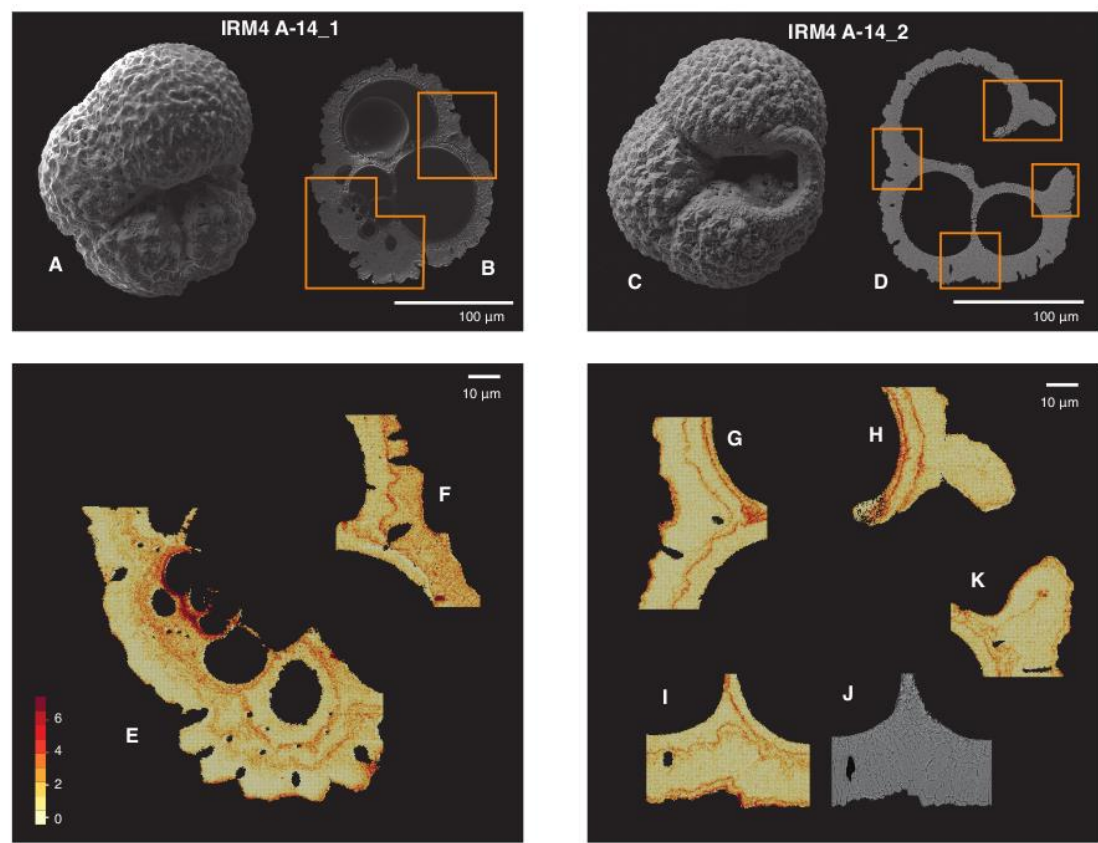


Figure 4

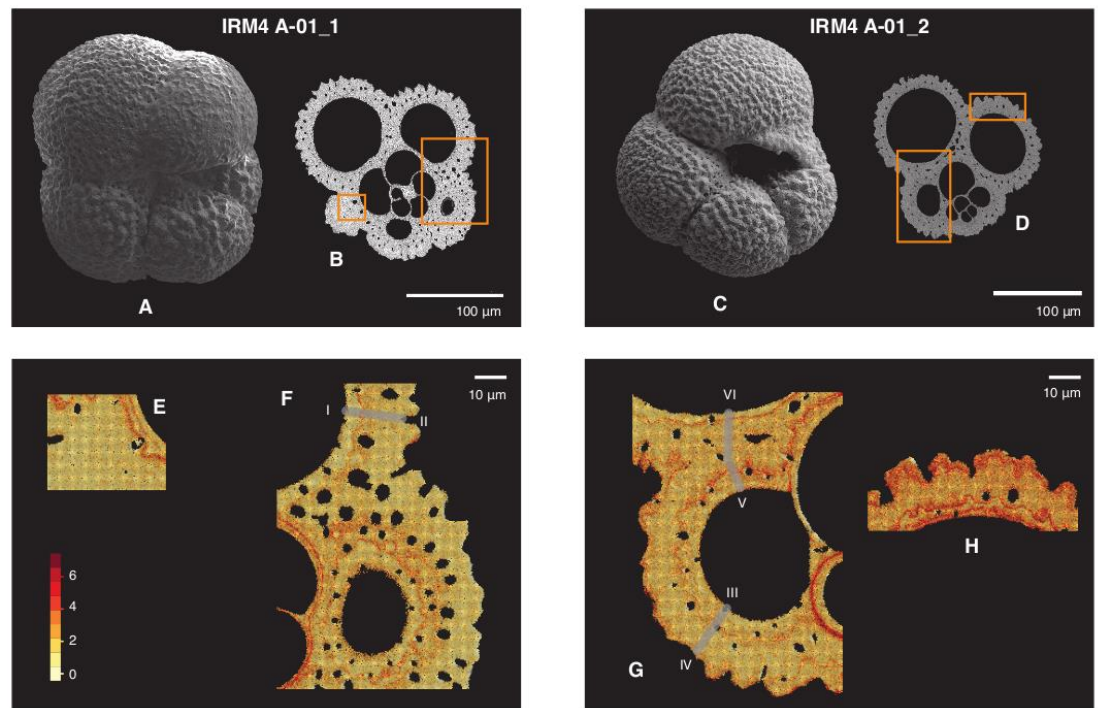


Figure 5

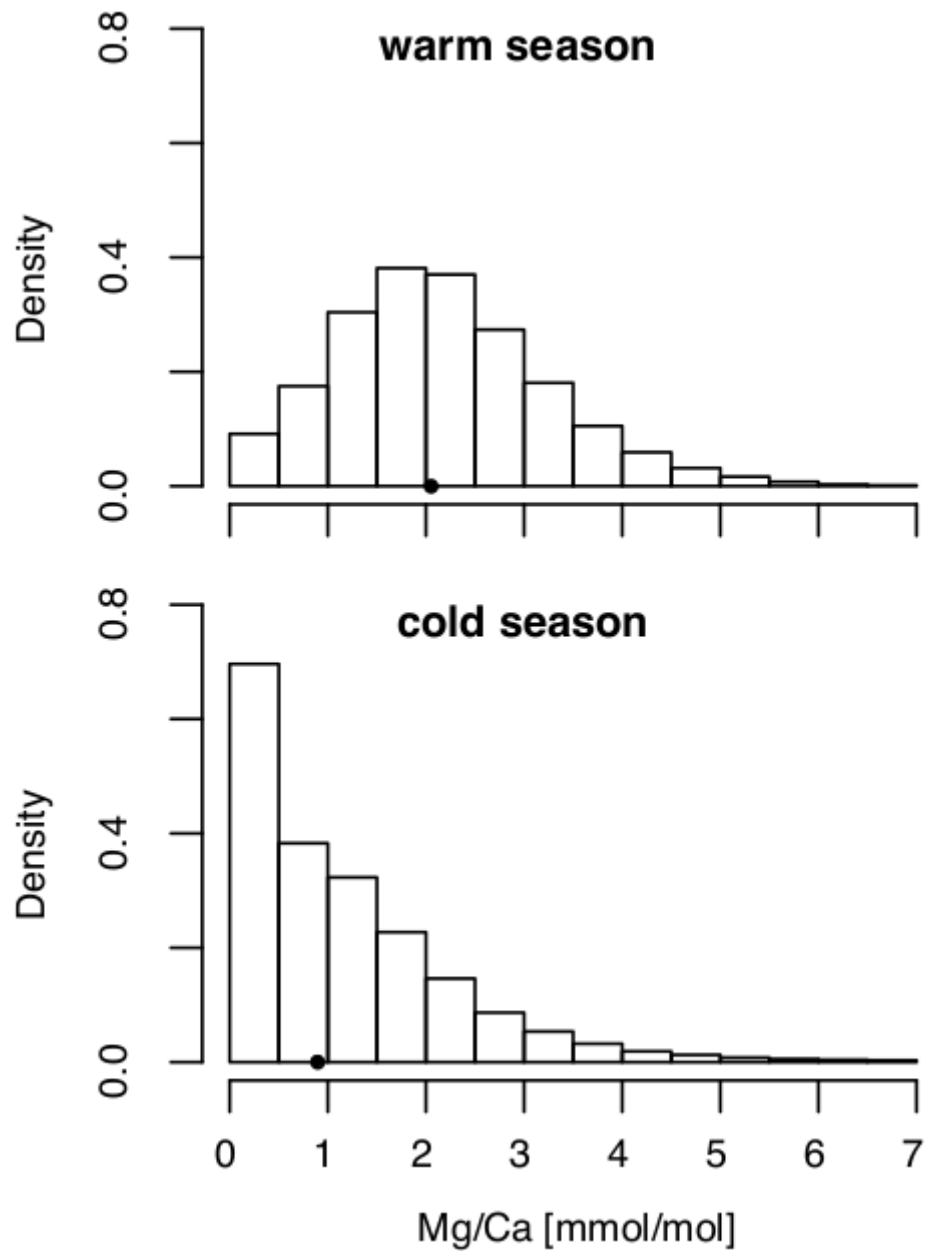


Figure 6

